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Clinical Evaluation of a Method for Detecting Superficial Transitional Cell Carcinoma of the Bladder by Light-Induced Fluorescence of Protoporphyrin IX Following Topical Application of 5-Aminolevulinic Acid: Preliminary Results

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Background and Objective: **In bladder cancer, conventional white light endoscopic examination of the bladder does not provide adequate information about the presence of "flat" urothelial lesions such as carcinoma in situ. In the present investigation, we examine a new technique for the photodetection of such lesions by the imaging of protoporphyrin IX (PpIX) fluorescence following topical application of 5-aminolevulinic acid (ALA).**
Study design/Materials and Methods: **Several hours after bladder instillation of an aqueous solution of ALA in 34 patients, a Krypton ion laser or a filtered Xenon arc-lamp was used to excite PpIX fluorescence. Tissue samples for histological analysis were taken while observing the bladder wall either by means of a video camera, or by direct endoscopic observation.**
Results: **A good correlation was found between the PpIX fluorescence and the histopathological diagnosis. On a total of 215 biopsies, 143 in fluorescent and 72 in nonfluorescent areas, all visible tumors on white light cystoscopy appeared in a bright red fluorescence with the photodetection technique. In addition, this method permitted to discover 47 unsuspected carcinomatous lesions on white light observation, among which 40% were carcinoma in situ.**
Conclusion: **PpIX fluorescence induced by instillation into the bladder of 5-ALA is an efficient method of mapping the mucosa in bladder carcinoma. Lasers Surg. Med. 20:402–408, 1997.**

Key words: **bladder neoplasms; transitional cell carcinoma; 5-aminolevulinic acid; fluorescence, LIF; photodetection; protoporphyrin IX**

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INTRODUCTION

Tumor multiplicity and/or the presence of concomitant "flat" urothelial carcinomatous foci of different grades, such as high-grade dysplasia or carcinoma in situ (CIS), are recognized as the most important predictive factors of local recurrence and progression in superficial bladder cancer [1]. As white light cystoscopy does not allow detection of these occult neoplastic lesions, bladder washing cytology, eventually supplemented by flow cytometry or static cytometry, is essential to achieve a correct staging of the disease. In spite of its high sensitivity, which can rise to 95% in presence of CIS [2], exfoliative cytology gives no precise information about the localisation and number of these carcinomatous foci. Various methods of labeling these occult urothelial neoplastic changes have been developed during the last 15 years. In vivo bladder mucosa staining with methylene-blue, previously considered a useful marker, proved to be inaccurate with a 68% false-negative rate for CIS [3]. Fluorescent tumor-localizing drugs such as tetracycline were not proven useful either for clinical applications because of their lack of specificity and their inability to be employed with conventional endoscopic instrumentation. Fluorescence studies correlated with histology on cystectomy specimens after hematoporphyrin derivative intravenous injection demonstrated the possible role of a photodynamic method to detect and localize dysplasia and CIS [4]. However, the long period of a possible risk of skin photosensitization after the drug administration certainly contributed to limit its clinical application. Recently, a first clinical experience [5] of photodynamic diagnosis of early stage disease in bladder cancer with an intravenous injection of the photosensitizer at a lower dosage (Photofrin II, 0.4 mg/kg, 48 hours prior to endoscopic observation) seemed to give interesting results in terms of sensitivity with a reduced risk of skin photosensitization. However, the value of the method to detect unsuspected carcinomatous foci was not mentioned, and this technique was finally abandoned probably because of its lack of specificity, the long incubation time of the photosensitizer, the insufficient contrast between malignant and benign tissue and the complexity of the procedure.

The high fluorescence contrast and intensity observed when treating cutaneous in situ tumors by photodynamic therapy using the endogenous photosensitizer, protoporphyrine IX (PpIX), induced by the topical application of 5-aminolevulinic acid (ALA) [6], suggested the use of the same approach to detect and treat epithelial tumors of different organs, such as the upper aerodigestive tract, the vagina, the cervix, the uterus, and the bladder.

In heme biosynthesis, ALA is a natural precursor of PpIX, an endogenous fluorescing photosensitizer. The first step of this cycle is the condensation of glycine and succinyl-CoA to yield ALA catalyzed by ALA synthase. The intracellular heme concentration regulates the production of ALA synthase. Since the conversion of PpIX to heme is a relatively slow process, the presence of an excess of ALA overrides this normal feedback regulation loop and leads to a temporary accumulation of PpIX in the mitochondria [7]. The reasons for the tumor selectivity of ALA-induced PpIX are not yet completely understood. Among the tentative explanations are: hyperactivity of malignant cells, which might be responsible of the higher uptake of ALA in tumor tissue as compared to benign tissue, increased activity of certain enzymes, which take part in the heme synthesis, and/or a reduction of ferrochelatase, which is necessary for the conversion of PpIX into heme. The latter could be caused by iron deficiency in the tumor. Iron consumption is effectively higher in malignant cells than in benign cells [8–10].

In mice, intraperitoneal administration of ALA induces high concentration of PpIX in the urothelium, but not in the underlying muscle [11]. These results demonstrate the "specificity" of this marker for epithelium. As was also shown in animal models, analysis of the fluorescence spectra measured in human bladder tumors shows large tumor/normal tissue fluorescence ratios which may even exceed 20:1 [12,13].

Following these observations, a new technique for the photodetection of neoplastic urothelial lesions by the imaging of the PpIX fluorescence induced by topical application of ALA has been developed [9]. In the study of the Munich group, the correlation of fluorescence and microscopic findings gave a sensitivity of 100% and a specificity of 68.5% and 26 malignant or precancerous lesions, missed during routine cystoscopy were detected only by the photodetection method.

We present here our initial experience of a similar technique with some modifications. The time of exposure of the photosensitizer (ALA) in the bladder of our patients was longer. Two different photodetection apparatus were used: a Krypton ion laser-based system and a filtered Xe-

non arc-lamp-based system. The bladder wall illumination was performed through the standard illumination optical fiber bundle of the cystoscope and not through a separate optical fiber. Finally, the spectral properties of the detection filters used were different.

MATERIALS AND METHODS

The following procedure was performed for every patient with superficial bladder cancer, as well as a few patients with infiltrative bladder cancers during a short hospitalisation for a conventional endoscopic treatment.

Fifty millilitres of a 3% solution of ALA, buffered with 5 ml phosphate-buffered saline (PBS) and adjusted to a pH of 5.3 with 7 ml sodium hydroxyde (1N), were instilled into the bladder through a 16 French Foley catheter ~7 hours (mean: 7 hr 22 min; range: 5 hr, 30 min-8 hr, 35 min) before photodetection. Patients were asked to keep the catheter closed during 4 hours (mean: 3 hr, 59 min; range: 2 hr, 55 min-4 hr, 45 min). Bladder urine contents were measured at the end of that topical application (mean: 289 cc; range: 100–600 cc) when the catheter was opened. The fluorescence excitation light and the resulting tissue fluorescence were transmitted through the optical fiber bundle of a 23.5 French STORZ cystoscope, thus leaving the passage for a cold biopsy forceps.

Two types of imaging photodetection apparatus have been used in this study. The first one was a Krypton ion laser-based system with excitation wavelengths at 407 nm and 413 nm. This apparatus performs the fluorescence detection in two spectral domains (in the red and in the green). This device was initially developed for cancer photodetection in the tracheo-bronchial tree with Photofrin II and has been described in detail elsewhere [14–16]. The second system was based on the use of a filtered Xenon arc-lamp (excitation wavelengths between 380 and 450 nm). The power of the fluorescence excitation light at the end of the cystoscope was between 10 and 25 mW for the Krypton ion laser system and between 150 and 480 mW for the STORZ Xenon arc-lamp system. In normal conditions of mucosal examination, the distal end of the cystoscope was placed at 2 cm of the bladder wall, so that the power density was typically ~3 mW/cm^2 and 65 mW/cm^2 for each system, respectively. The Krypton ion laser system had a commutation device that allowed passage at any time from white light observation to laser light-induced fluorescence detection and back. Such a switch was not available for the STORZ Xenon arc-lamp system.

With the Krypton ion laser system, tissue fluorescence was separated by a dichroic mirror into two images (one below and one above 580 nm). The background image was then passed through a bandpass filter at 510–580 nm giving the “green” fluorescence image. The foreground image containing the PpIX fluorescence was passed through a long-pass filter $\lambda > 600$ nm to give a “red” fluorescence image. The fluorescence emission peak of the PpIX is at 635 nm. Both the red and green images are amplified by ~10^4 times and displayed on a colour (RGB) monitor. The spatial resolution of both systems is identical to that of a conventional color camera for endoscopic use, i.e., 470 horizontal TV-lines and 450 vertical TV-lines.

In the lamp system, the high power of the filtered Xenon arc enabled a direct visual observation of the bladder wall fluorescence through the cystoscope using simply a long-pass filter ($\lambda >$ 520 nm). This filter transmits the red and cuts out most of the blue reflected excitation light. Absorption of the remaining blue and/or green excitation light by blood vessels permits identification of the site on the bladder wall. Photographs of the bladder wall under fluorescence were taken with a RICOH camera with a Kodak Wratten N° 9 filter inserted between the 70 mm camera lens and the endoscope eyepiece. The exposure time was 1/2–2 seconds. Sensitive films were required to obtain a satisfactory image, i.e., Kodak Ektachrome P 1600x films, developed at 1600 ASA.

Bladder biopsies were taken during the photodetection.

Patients

Since February 1994, 34 patients, 21 men and 13 women, were enrolled in this study. The mean age was 67.9, range 44–84 years. The bladder cancer was superficial in 28 patients, i.e., pTa, pTis, pT1 in the TNM classification, and invasive in 6 patients. Thirteen patients had never been operated for a bladder cancer before the procedure; 21 patients previously had one (9 patients), two or iterative (12 patients) TURB (transurethral resection of the bladder).

A bladder topical chemotherapy or immunotherapy with BCG was added to the previous surgical treatment for 13 patients. Thirty-two patients had a bladder urinary cytology at the time

TABLE 1. Correlation Between Histopathological Diagnosis and Fluorescence*

	Presence of carcinoma	Absence of carcinoma
Positive fluorescence	97 (47[a])	46
Negative fluorescence	12	60

*Total number of biopsies: 215. Rate of false negative: 11%. Rate of false positive: 43%.
[a]Number of invisible carcinoma foci detected by fluorescence only.

when the catheter was placed the day before the procedure.

The 215 biopsies, which represent a mean of 6.3 biopsies per patient, were taken during fluorescence photodetection procedure; 143 in fluorescing areas and 72 in nonfluorescing areas. Four of the 72 nonfluorescent sites displayed nonspecific signs of mucosal inflammation on white light endoscopic examination. Histologic examination was performed independently by two pathologists, who were not informed about the endoscopic findings. Papillary and nonpapillary urothelial carcinomas were graded and staged according to the W.H.O. 1973 classification [17] and the UICC/AJC 1992 system [18], respectively. Flat intraepithelial neoplastic lesions were graded according to criteria of Nagy et al. [19] and classified as grade 1 (mild dysplasia), grade 2 (moderate dysplasia), grade 3 (marked dysplasia), and in situ carcinoma.

RESULTS

No systemic or local side effect was noted following ALA bladder instillation, before and after the photodetection procedure. In Table 1, the presence or absence of cancer cells as indicated by histopathology is correlated with the fluorescence findings. Essentially identical results were obtained with both the Krypton laser-based and the Xenon lamp-based systems. Therefore, results obtained with both systems are presented together.

All papillary exophytic pediculated or sessile tumors and all planar tumors appearing clearly malignant during white light endoscopic bladder wall examination fluoresced strongly in the red. In addition, the fluorescence precisely demarcated the tumor outline. It was possible to operate in the bladder under direct vision when using the filtered Xenon light source. With a long pass filter ($\lambda > 520$ nm) on the eyepiece of the cystoscope, the bladder wall appeared in pale green, and the vessels of the lamina propria were finely delineated and were somewhat darker. Spots of invisible foci of "flat" early carcinoma as well as visible papillary tumors appeared in a bright red fluorescence. (Figs. 1–3).

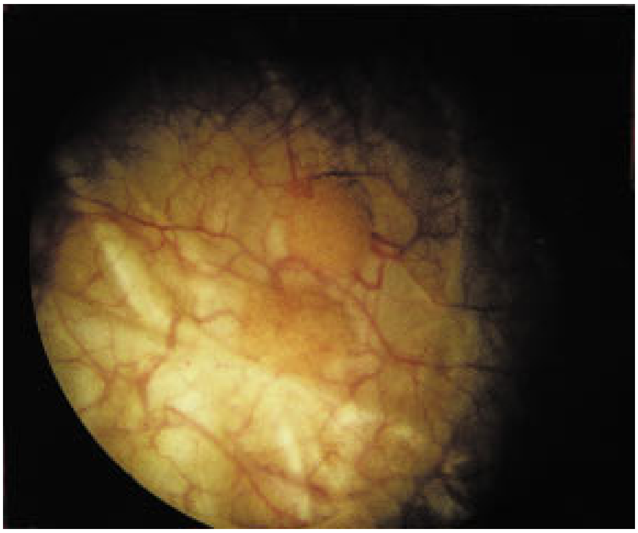
Histopathological diagnosis and fluorescence response were correlated. This fluorescence method considerably enhances the detection of light to moderate dysplasia and particularly of CIS. Indeed, 47 foci of carcinoma in 18 patients, which were invisible under white light observation, could be detected by fluorescence, whereas only 12 "false negative" in 9 patients were noticed (see Table 2). In addition, among these 47 foci of carcinoma detected only by fluorescence, > 40% (19 in 5 patients) were CIS, whereas only one CIS was missed, probably because it was situated on an ulcer border.

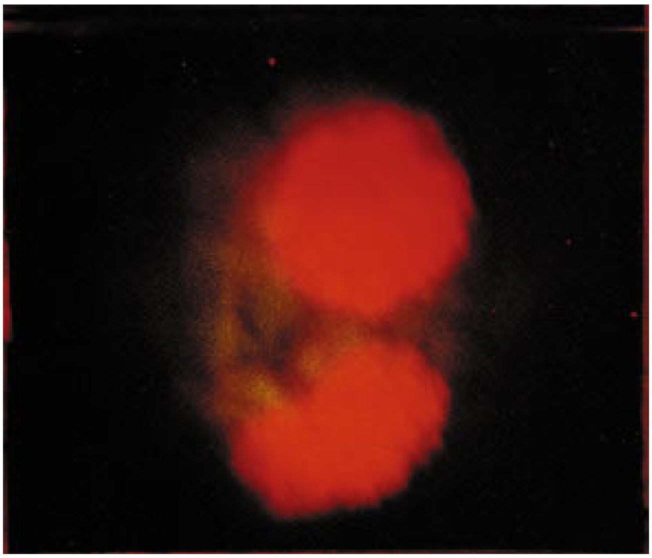
Table 3 lists the histopathological diagnosis of the 46 "false positive results" in 18 patients. These results indicate that for most of the patients, there was a clear histopathologically established reason. Twenty-four lesions appeared visible as oedematous, erythematous, erosive, or scar formations of the bladder wall under white light exposition, whereas 22 false positive fluorescent sites were found on an apparently healthy mucosa. Only one patient had false negative and false positive results.

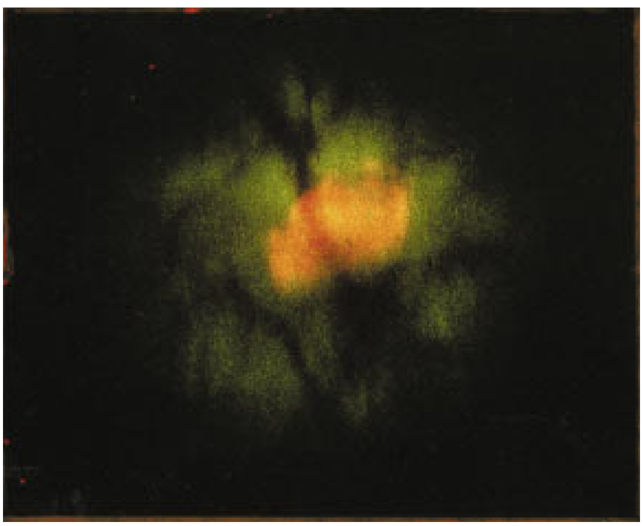
DISCUSSION

In the Canton of Vaud in Switzerland, the incidence of superficial bladder cancer is ~18.6/100,000/year in male patients and 4.2/100,000/year in female patients, which represents a ratio of 4:1 [20]. This incidence rate of the disease is similar in the United States [21]. According to the literature [22], the individual risk of progression, which reflects the highly variable natural history of this disease, varies from 4% to 45%. As noted above, the best chances to cure superficial bladder cancer are related to the presence of predictive factors for local recurrence and progression. In fact, the characterisation of the dysplastic changes on the bladder mucosa seems to be the best way to evaluate the prognosis of the disease.

No systemic reaction was observed following bladder instillation of ALA or illumination with violet light. Furthermore, fluorescence intensity distributions measured in biopsies by fluorescence microscopy confirmed the selective accumulation of PpIX in the urothelium as compared to the stroma, as well as the higher fluorescence in-







Figs. 1–3.

TABLE 2. Histopathological Diagnosis of 47 Invisible Foci of Carcinoma Detected by Fluorescence Compared to 12 False-Negative Results

Histopathological diagnosis	Fluorescence positive[a]	Fluorescence negative "false negative"[a]
Dysplasia G1	14 (9)	4 (4)
Dysplasia G2	8 (2)	5 (2)
Dysplasia G3	2 (1)	0
CIS	19 (5)	1 (1)
Carcinomatous chorionic lymphangitis	4 (1)	1 (1)
pTaG1	0	1 (1)
Total:	47 (18)	12 (9)

[a]Number of patients in parens.

tensity in malignant cells as compared to benign cells [23].

With the exception of a slightly higher number of false-negative responses found in our study, the results presented here are quite similar to those obtained by the group in Munich.

A satisfactory correlation between tissular fluorescence response and histopathological diagnosis is clearly demonstrated. As the bladder wall was not systematically biopsied at random, a precise estimation of the sensitivity cannot be derived from the present results. However, considering the 47 invisible foci of carcinoma detected by fluorescence, the sensitivity of this method appears to be twice as good as the one of conventional white light cystoscopy.

The rate of false-positive responses (43%) is acceptable, in particular considering the numerous previous surgical treatments and local chemo- or immunotherapy undergone by two-thirds of the patients. A higher cellular turnover in the bladder wall due to scarring or residual inflammations could partly explain a large fraction of the false-positive responses. Quantitative excitation and emission fluorescence spectroscopy of endogenous fluorochromes is now underway to try to

Fig. 1. Conventional endoscopic appearance of two small papillary tumors Ta, G1. Estimated diameter: 7 mm for each papilloma.

Fig. 2. Fluorescence appearance of the same two tumors as in Figure 1. Exposure time: 2 sec. Estimated diameter: 7 mm for each papilloma.

Fig. 3. Example of a carcinoma in situ detected by fluorescence. Exposure time: 1 sec. Estimated size: 6 × 4 mm.

TABLE 3. Histopathological Diagnosis of 46 False Positive Results Distributed According to Their Macroscopic Aspect on "White Light" Cystoscopy in 18 Patients

"White light" cystoscopy: unspecific visible mucosal alterations		"White light" cystoscopy: apparently normal mucosa	
Abrasion	1	Abrasion	4
Scar formations	5	Metaplasia	1
Granulomatous inflammation	4		
Cystitis	3		
Follicular cystitis	9	Follicular cystitis	3
Normal urothelium	1	Normal urothelium	12
Hyperplasia	1	Hyperplasia	2
Total:	24		22

improve the discrimination between benign and malignant tissues. It is of interest to note that beside these false positives caused by nonmalignant tissue changes observed in histopathology, any oblique illumination of the mucosa can result in false positives if the distal end of the endoscope is positioned too close to the bladder wall. This effect, also observed during a conventional "white light" endoscopy, which makes the tissues appear red, is due to the differential propagation of green and red fluorescence light in the tissues.

Compared to the Krypton ion laser-based system designed in Lausanne [14], the use of the new Storz Xenon violet light source simplifies the procedure. It enables the operator to examine the bladder wall directly in real time without any auxiliary device. Both techniques delineate precisely the extent of a solitary tumor and give information about the possible presence of additional epithelial dysplastic changes. This is particularly useful in case of a diffuse carcinoma in situ. Thus this fluorescence imaging technique helps to biopsy the apparently normal urothelium at the right place and most likely eliminates the necessity of blind random biopsies, whose usefulness remains controversial in the prognostic evaluation of the disease [24]. Hence, PpIX-induced fluorescence with topical bladder instillation of ALA is an efficient, quick, and easy method of mapping the mucosa in bladder cancer. The prognostic value of the dysplastic changes, other than in situ carcinoma, revealed on bladder mucosa will be elucidated, as well as the long-term effect on the progression of the disease of bladder tumor resection under ALA-induced PpIX fluorescence.

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